Antigen-presenting B cells and helper T cells cooperatively mediate intravirionic antigenic competition between influenza A virus surface glycoproteins

(vaccine/neuraminidase/hemagglutinin/immunity)

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ABSTRACT Parenteral vaccination of BALB/c mice primed by infection with H3N2 variants of influenza A virus results in a reduced production of N2 antibody in response to homologous (H3N2) vaccine compared with the response to an H7N2 vaccine equal in N2 immunogenicity. We now have studied the interaction *in vitro* of purified splenic B and T lymphocytes from variably immunized mice to ascertain the cellular basis of the hemagglutinin (HA)-influenced antibody response to neuraminidase (NA). Assay of the proliferative response of T cells in B/T-cell mixtures stimulated by H3N1 (HA-specific) and H6N2 (NA-specific) reassortant (recombinant) viruses *in vitro* has enabled us to differentiate cellular responses to HA and NA antigens. Using a factorial design in analysis of B/T-cell mixtures, we have shown that: (*i*) intravirionic HA is dominant over NA in both B- and T-cell priming; (*ii*) an increase in H3-specific B cells occurs in mice administered boosters of H3N2 vaccine, and an increase in N2-specific B cells occurs in those given a booster of H7N2 vaccine; and (*iii*) memory B cells function as antigen-presenting cells and interact with memory helper T cells in the mediation of intravirionic HA–NA antigenic competition in favor of HA. The damping of response to the NA antigen in favor of HA with reinfection prohibits balanced immunologic response to the two antigens. The present studies define further the complex immunology of influenza virus infection.

Hemagglutinin (HA) and neuraminidase (NA), surface glycoproteins of influenza A virus, are both immunogenic; however, the immune response to either antigen is influenced by priming to the other (1). Antigenic competition between these surface glycoproteins is manifest in natural infection as a suppression of the anti-NA immune response (1). Suppression can be avoided by presentation of NA in association with an HA not previously encountered by the experimental animal's immune system (2, 3). Adoptive transfer experiments have shown that reduced generation of NA-specific T cells is at least partially responsible for modulation of this antigenic competition in favor of HA (3). However, there is evidence that intermolecular antigenic competition may be a side effect of B cell–T cell cooperation (4–7). Scherle and Gerhard (7) have shown that the B cell–T cell collaboration that results in an antiviral immune response requires a cognate (i.e., direct) T cell–B cell interaction, whether or not the determinants recognized by the helper T cells and B cells are located on the same viral protein or on different proteins within the viral particle. Therefore, one hypothesis that would explain the antigenic competition between influenza virus surface glycoproteins takes cognizance of the ability of B cells to bind, process, and present antigen to T cells. This hypothesis assumes that primary immunization with influen-

za virus leads to preferential expansion and affinity maturation of B cells specific for HA, the most abundant surface antigen (8). When the host is reimmunized with the same virus, HA-specific B cells would preferentially bind the virus and process and present it to T cells specific for one of the several influenza viral proteins. The anti-NA response would be blocked by competition unless the NA were presented in association with a different HA on the virion (2). We have tested this hypothesis by measuring the proliferative response of purified T cells stimulated by influenza virus antigens presented by purified B cells.

MATERIALS AND METHODS

Influenza A virus strains used for infection or vaccination were as described (3). Antigenically hybrid, "reassortant" influenza A viruses (viruses derived from gene reassortment by coinfection) H3N1 (A/Hong Kong/1/68–PR/8/34) and H6N2 (A/Turkey/Mass/76–Aichi/2/68) purified on sucrose gradients were UV-inactivated to a residual 50% egg infective dose (EID$_{50}$) of $<10^{-1.3}/0.2$ ml and then were used in *in vitro* proliferation assays.

Animals. Influenza-immunized BALB/c female mice (The Jackson Laboratories) from a previous study (3) were donors of B and T cells used in the present study.

Infection and Vaccination Procedure. The procedure of sequential infection with heterovariant H3N2 influenza A viruses has been described in detail (2). Vaccination procedures were as described by Johansson et al. (3). After sequential H3N2 infections, animals were injected with either phosphate-buffered saline (PBS) or B/Lee, A/H3N2, or A/H7N2 influenza virus vaccines. The immunization schedule and antibody responses from this study are presented in brief in Table 1.

Preparation of T-Cell-Enriched Populations from Spleen Cells. Purified T-cell populations were obtained 3 months after final immunization from the spleens of freshly killed animals as described (3). Cytofluorometric analysis with fluorescein-labeled anti-Thy-1.2 monoclonal antibody showed that >97.5% of these cells were T cells.

Preparation of B-Cell-Enriched Populations from Spleen Cells. Splenocytes used as a B-cell source were brought to a volume of 3 ml in Dulbecco's modified essential medium (DMEM) with 5% fetal calf serum and then were placed in 100-mm Petri dishes (Fisher) coated with a 1:10 dilution of purified rabbit anti-mouse immunoglobulin antibody and normal rabbit serum (9). The dishes were gently rocked for 70 min at 4°C. To recover adherent cells, the plate was filled with 20–25 ml of PBS containing 1% fetal calf serum, and the entire surface of the plate was flushed by using a Pasteur pipette. Cells were incubated with a 1:30 dilution of anti-Thy-1.2 monoclonal antibody at 4°C for 30 min and then with a 1:16

Abbreviations: HA, hemagglutinin; NA, neuraminidase.
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Table 1. Immunization protocol and serologic response of mice to sequential infection and vaccination with influenza viruses

Mouse group	Serologic response* on day PI1 (postinfection 1)								
	Titer after infection				Vaccine injection i.p. (day 63 PI1)	Titer after injection			
	1[†] (day 42 PI1)		2[‡] (day 63 PI1)			(day 70 PI1)		(day 77 PI1)	
	NI	HI	NI	HI		NI	HI	NI	HI
1	1.0	1.0	1.0	1.0	Mock	1.4	1.0	1.1	1.0
2	4.8	2.5	4.5	1.9	Mock	5.6	2.8	7.0	2.6
3	4.7	2.3	5.0	1.9	H3N2[§]	7.1	6.6	11.0	7.9
4	4.7	2.3	4.9	2.1	H7N2[¶]	8.0	4.5	14.6	5.1
5	5.2	2.4	5.4	2.1	B/Lee	5.2	3.1	6.4	3.8
Vaccine standardization group									
A					H3N2	1.6	0.9	3.2	2.0
B					H7N2	1.5	1.0	2.9	1.0

*NA inhibition (NI) antibody titer (H3N1 test virus) is expressed as the reciprocal of the geometric mean titer; HA inhibition (HI) antibody titer (H3N1 test virus) is expressed as the reciprocal of the geometric mean titer.

[†] Aerosol exposure to A/Hong Kong/1/68(H3N2) virus (groups 2–5), group 1 being mock-infected (day 0).

[‡] Intranasal inoculation of A/Philippines/2/82(H3N2) (groups 2–5), group 1 being mock-infected (day 42).

[§] A/Hong Kong/1/68(H3N2).

[¶] A/Equine/Prague/1/56–Aichi/2/68(R)(H7N2).

dilution of rabbit complement at 37°C for 30 min. Cytofluorometric analysis of these cells showed that 95.6% stained positively with tetramethylrhodamine-labeled rabbit anti-mouse Ig antibody.

T-Cell Proliferative Response. Purified B cells from each experimental group were incubated with 100 hemagglutinating units of antigenically hybrid reassortant virus, H3N1 or H6N2, for 8 hr and then γ-irradiated (2000 rads), yielding HA (H3)-specific or NA (N2)-specific activated B cells. Virus-stimulated B cells (2×10^5 per well) and T cells (4×10^5 per well) were cocultured in a total volume of 200 μl per well. These cultures were maintained at 37°C in 5% CO_2/95% air for 36 hr and then pulsed with 1 μCi of [^{3}H]thymidine per well. After 18 hr of incubation with label, cells were harvested and radioactivity was measured. Cell cultures were maintained in DMEM containing 2-mercaptoethanol (5 μM) and 10% fetal calf serum. In the absence of virus, the T-cell proliferative response of purified B/T-cell mixtures from unimmunized animals was comparable to that found with unfractionated splenocytes in other studies ($\approx$1500 cpm). However, in the presence of virus, these background levels were 10–30 times higher, possibly reflecting nonspecific B-cell activation by the panning procedure used in B-cell isolation. The use of virus reassortants (H6N2 or H3N1) containing identical internal proteins (from A/PR/8/34) and only one of the surface glycoproteins to which the experimental animals had been previously primed enabled us to differentiate cellular responses to each surface antigen. After purification, each group of purified T cells was paired with each group of antigen-stimulated B cells in a standard factorial design (Fig. 1).

RESULTS

Comparative Primary Immunogenicity of H3N2 and H7N2 Vaccines as Measured by Splenocyte Proliferation Response. Splenocytes from unprimed mice injected with either H3N2 or H7N2 vaccine proliferated to equivalent levels when stimulated *in vitro* with H6N2 virus (Table 2), confirming previous findings that H7N2 and H3N2 inactivated vaccines are equivalent in primary N2 immunogenicity. However, when H3N1 was used as the stimulator virus, only spleno-
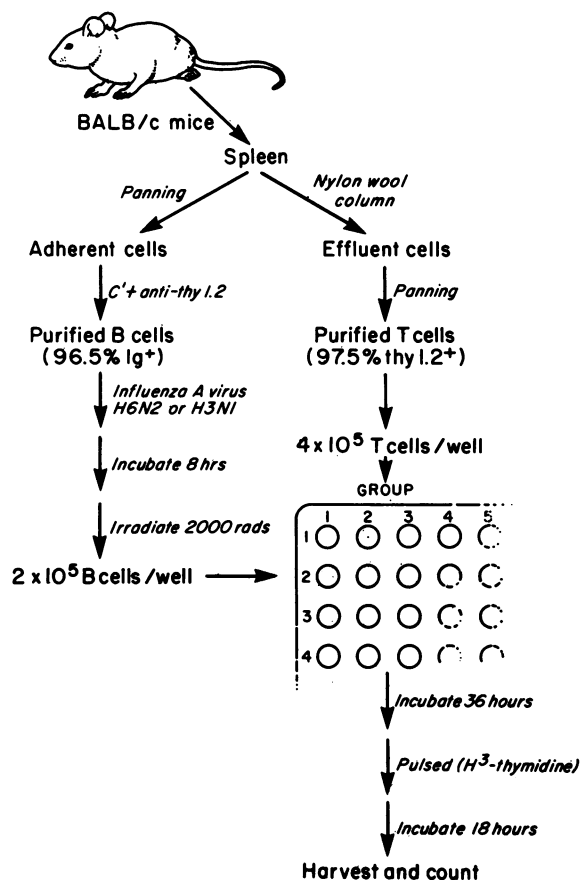

FIG. 1. Purified cell populations for *in vitro* proliferation assays were obtained as outlined above. See text for details.

cytes from animals injected with H3N2 vaccine proliferated significantly (Table 2), although splenocytes from H7N2-vaccinated mice did proliferate above background levels in response to H3N1 virus. Low-level crossreactivity of H7 and H3 has been noted (2, 3, 10).

Priming Effects of Sequential Infection: Segregation of B- and T-Cell Responses by Study of *in Vitro* Antigenic Stimulation of B/T-Cell Mixtures. Stimulation of B/T-cell mixtures from unprimed (group 1) mice with either the H3N1 or H6N2 virus resulted in equivalent T-cell proliferative responses (Table 3, experiments 1 and 8).

The priming of cells to both external antigens of the infecting H3N2 viruses is shown by comparison of experiments 6 and 7 (H3-specific recall) and experiments 13 and 14 (N2-specific recall) with proliferative responses of unprimed cells (experiments 1 and 8).

The immunodominance of H3 over N2 is seen in comparisons of proliferative response to H3N1 stimulation (experiments 6 and 7) with H6N2 stimulation (experiments 13 and

Table 2. Proliferation of splenocytes from unprimed mice injected with H3N2 or H7N2 vaccine

In vitro stimulator virus*	[^{3}H]Thymidine incorporation after infection,[†] cpm ± SEM	
	H7N2	H3N2
H6N2	40,844 ± 1817	38,609 ± 2202
H3N1	9,095 ± 1500	74,773 ± 1129
None	1,868 ± 304	1,399 ± 119

*One hundred hemagglutinating units of virus were used as the stimulus.

[†] Values are mean cpm ± SD of triplicate cultures.

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Table 3. Interaction of B and T cells from sequentially infected and unimmunized mice

	B cells		T cells			cpm ×	
Exp.	Mouse group	In vivo primed	Mouse group	In vivo primed	Test virus	10^{-3}/ min*	Rank¶
1	1	None†	1	None†	H3N1	49	E
2	1	None†	2	H3N2‡	H3N1	97	C
3	1	None†	5	H3N2‡ B/Lee§	H3N1	100	C
4	2	H3N2‡	1	None†	H3N1	95	C
5	5	H3N2‡ B/Lee§	1	None†	H3N1	97	C
6	2	H3N2‡	2	H3N2	H3N1	200	A
7	5	H3N2‡ B/Lee§	5	H3N2 B/Lee§	H3N1	205	A
8	1	None†	1	None†	H6N2	48	E
9	1	None†	2	H3N2‡	H6N2	94	C
10	1	None†	5	H3N2‡ B/Lee§	H6N2	85	C
11	2	H3N2‡	1	None†	H6N2	60	D
12	5	H3N2‡ B/Lee§	1	None†	H6N2	69	D
13	2	H3N2‡	2	H3N2‡	H6N2	105	B
14	5	H3N2‡ B/Lee§	5	H3N2‡ B/Lee§	H6N2	117	B

*T-cell proliferation as measured by [^{3}H]thymidine incorporation; values are mean cpm × 10^{-3}/min of triplicate cultures. Replicates were within ± 12% of the mean, so SDs are omitted.
†Animals given sterile PBS.
‡Sequential infection by H3N2 heterovariant viruses.
§Injection i.p. of virus.
¶Tukey test ($\alpha = 0.05$) subsequent to analysis of variance ($P < 0.0004$) placed significantly different groups in rank order as indicated.

Table 4. Rank order of mean T-cell and B-cell proliferation response to N2-specific and H3-specific antigen stimulation

Exp. A with stimulator virus H6N2			Exp. B with stimulator virus H3N1		
Mouse group	Mean cpm*	Rank†	Mouse group	Mean cpm*	Rank†
		T cells			
1	98,872	D	1	95,049	C
2	143,151	C	2	184,892	B
3	209,310	B	3	274,831	A
4	279,894	A	4	183,784	B
5	137,846	C	5	186,280	B
		B cells			
1	114,401	D	1	94,526	C
2	138,055	C	2	192,017	B
3	181,783	B	3	255,147	A
4	299,095	A	4	192,691	B
5	145,739	C	5	190,455	B

*Mean cpm of [^{3}H]thymidine incorporation in all cultures containing T cells in experiment A (or B cells in experiment B) from a given group. Each value represents 15 replicates [five B-cell groups in experiment A (or T-cell groups in experiment B) from three cultures per group = 15].
†Tukey test ($\alpha = 0.05$) subsequent to analysis of variance ($P < 0.0001$) placed significantly different groups in rank order as indicated.

Proliferative Response of T Cells Stimulated by H3N1: Animals Vaccinated with H3N2 Show the Greatest Response. When H3N1 was used as stimulating test virus (Table 4, experiment B), a different pattern of difference among groups was found. T cells from groups of animals injected with H3N2 virus after sequential infection with H3N2 variants had the greatest *in vitro* response to H3N1 stimulator virus (group 3). There were no significant differences among proliferation responses of T cells taken from animals sequentially infected and then injected with H7N2 (group 4), B/Lee (group 5), or PBS (group 2), although these groups were significantly different from group 3 (highest response) and group 1 (mock-infected; lowest response). The pattern of response among B-cell groups from these mice was identical to the pattern found with T-cell groups.

Comparative Responses of Primed and Unprimed T Cells Stimulated by Naive B Cells: A Measure of T-Cell Memory. By culturing T cells from each group with naive B cells, we recreated *in vitro* a cellular situation comparable to the transfer of purified T cells to naive athymic mice (3) (Table 5). When T cells from each infection/vaccination group were exposed to naive B cells incubated with H6N2 virus, T cells from H7N2-vaccinated mice (group 4) had a significantly higher response than did cells of any other group. The T-cell response of group 3 (H3N2) was significantly greater than responses of cells of groups 2 (PBS) and 5 (B/Lee-immunized) but was significantly less than that of group 4 (H7N2) T cells. Unprimed T cells of group 1 (mock-infected) had the least response to stimulation by H6N2 virus.

When H3N1 virus was used in this assay, T cells from animals injected with H3N2 virus after sequential infection (group 3) had the highest response. The proliferation responses to H3N1 by T cells from group 4 (H7N2), group 5 (B/Lee), and group 2 (PBS) cannot be separated statistically. Again, cells of group 1 (mock-infected) had the lowest *in vitro* response to stimulating virus.

Comparative Efficacy of Primed and Unprimed B Cells in Stimulating Proliferative Response in Naive T Cells. To determine the role of B cells in the response of sequentially infected and vaccinated mice, we cocultured virus-stimulated B cells from each group with naive T cells (Table 6). With H6N2 test virus, the highest proliferation response was found

14) of cells from the same infected mice. This HA-skewed immune response can be explained as the result of greater B-cell priming. A greater proliferative response in "naive" T cells (from mock-infected animals, group 1) was induced by primed B cells stimulated by H3N1 than by primed B cells stimulated by H6N2 virus (experiments 4 and 5 compared with 11 and 12). In contrast, T cells from infected animals exhibited no difference in proliferative response to H3- and N2-specific stimulation when mixed with naive B cells (compare experiments 2 and 3 to 9 and 10). It is interesting that group 2 and group 5 B cells were only slightly better at presenting H6N2 to naive T cells than were group 1 (unprimed) B cells (Table 3, experiments 11 and 12). This shows that very little B-cell memory was established for N2, even though both groups were infected twice with viruses containing N2. These results are consistent with our original hypothesis that little expansion of NA-specific B cells occurs when NA is repetitively presented in the context of the same HA, and the results also suggest that internal proteins elicit little or no B-cell memory.

Proliferative Response of T Cells Stimulated by H6N2: Superior Proliferation in H7N2-Vaccinated Animals. Among T-cell groups, those animals injected with H7N2 virus after sequential infection (Table 4, experiment A, group 4) had the greatest T-cell proliferative response to H6N2, and animals that received H3N2 vaccine (group 3) had the next highest response. The T-cell proliferative responses of animals injected with PBS (group 2) or B/Lee virus (group 5) were significantly lower than that of group 3 or 4 but were not significantly different from each other. Differences among proliferative responses induced by the various B-cell groups reflect patterns identical to those found with T-cell groups.

Table 5. Comparative efficacy of primed and unprimed T cells stimulated by naive B cells from mock-infected animals (group 1)

| T-cell mouse group | *In vivo* priming | [³H]Thymidine incorporation with test viruses* | | | |
| | | H6N2 | | H3N1 | |
		cpm × 10⁻³/min	Rank†	cpm × 10⁻³/min	Rank†
1	None	48	D	49	C
2	H3N2‡	94	C	97	B
3	H3N2‡ H3N2§	119	B	146	A
4	H3N2‡ H7N2§	194	A	98	B
5	H3N2‡ B/Lee§	85	C	100	B

*T-cell proliferation as measured by [³H]thymidine incorporation; values are mean cpm × 10⁻³/min of triplicate cultures. Replicates were within ± 12% of the mean, so SDs are omitted.
†Tukey test ($\alpha = 0.05$) subsequent to analysis of variance ($P < 0.0001$) placed significantly different groups in rank order as indicated.
‡Sequential infection by H3N2 heterovariant viruses.
§Injection i.p. of virus.

in cultures containing group 4 B cells. This proliferation response was significantly greater than the next highest response (group 3). Proliferation responses from groups 2 and 5 could not be separated statistically. However, when H3N1 virus was used to stimulate B cells from each group, the greatest proliferation of naive T cells occurred when B cells from group 3 were used. The proliferative response of cultures containing B cells from group 3 was significantly higher than the responses in cultures containing B cells from groups 4, 2, or 5 among which there were no statistically significant differences.

HA–NA Antigenic Competition Is Demonstrated by Antigen-Specific *in Vitro* Proliferation in B/T-Cell Mixtures. Purified B cells and purified T cells from vaccine-boosted immunization groups 3 and 4 were mixed after B cells had been exposed to test virus. The data summarized in Table 7 show that, in mixtures of group 3 T cells and group 3 B cells (experiment 1), T cells proliferated more than twice as well as did group

Table 6. Comparison of primed and unprimed B cells in stimulating proliferation response in naive T cells from mock-infected animals (group 1)

| B-cell mouse group | *In vivo* priming | [³H]Thymidine incorporation with test viruses* | | | |
| | | H6N2 | | H3N1 | |
		cpm × 10⁻³/min	Rank†	cpm × 10⁻³/min	Rank†
1	None	48	D	49	C
2	H3N2‡	60	C	95	B
3	H3N2‡ H3N2§	92	B	162	A
4	H3N2‡ H7N2§	223	A	100	B
5	H3N2‡ B/Lee§	69	C	97	B

*T-cell proliferation as measured by [³H]thymidine incorporation; values are mean cpm × 10⁻³/min of triplicate cultures. Replicates were within ± 12% of the mean, so SDs are omitted.
†Tukey test ($\alpha = 0.05$) subsequent to analysis of variance ($P < 0.0002$) placed significantly different groups in rank order as indicated.
‡Sequential infection by H3N2 heterovariant viruses.
§Injection i.p. of virus.

Table 7. Intra- and intergroup comparisons of B cell–T cell interaction in response to *in vitro* stimulation

| Exp. | Mouse group cells | | *In vivo* priming | [³H]Thymidine incorporation with *in vitro* viral stimulus,* cpm × 10⁻³/min | |
	B	T		H3N1	H6N2
1	3	3	H3N2† H3N2‡	481	212
2	4	4	H3N2† H7N2‡	199	439
3	3	4	Mixed§	210	189
4	3	1	H3N2† H3N2‡	162	92
5	1	3	H3N2† H3N2‡	146	119
6	4	3	Mixed§	269	330
7	4	1	H3N2† H3N2‡	100	223
8	1	4	H3N2† H7N2‡	98	194

*T-cell proliferation as measured by [³H]thymidine incorporation; values are mean cpm × 10⁻³/min of triplicate cultures. Replicates were within ± 12% of the mean, so SDs are omitted.
†Sequential infection by H3N2 heterovariant viruses.
‡Injection i.p. of vaccine virus.
§Group 3 donors were sequentially infected by H3N2 virus and then injected i.p. with H3N2; group 4 donors were similarly infected but were injected with H7N2 virus.

4 T cells in contact with group 4 B cells (experiment 2) in response to *in vitro* stimulation with H3N1 virus. Conversely, mixtures of B and T cells from group 4 (H7N2 vaccine) responded more than twice as well to H6N2 virus as did cells from group 3. Although the first observation is explicable on the basis that group 4 animals had had less H3 priming, groups 3 and 4 had had equal exposure to the N2 antigen. These results are congruent with our previous demonstration of HA–NA antigenic competition based on antibody response (1, 2) and provide evidence that the effect is mediated by B cell–T cell interaction. The predominant role of B cells as antigen-presenting cells was most clearly seen when primed B cells from these two groups presented either H6N2 or H3N1 virus to naive T cells (experiments 4 and 7). An additional point of interest is that the greatest response to *in vitro* stimulation with H3N1 virus was in mixtures of group 3 B and T cells. Also, when group 4 B cells were paired with group 4 T cells, proliferative response to H6N2 was superior to that shown with intergroup cell mixtures (experiments 3 and 6).

DISCUSSION

In previous studies we demonstrated that BALB/c mice sequentially infected with two antigenic variants of H3N2 influenza virus and then vaccinated with the first H3N2 strain developed less antibody to the viral (N2) NA by a factor of 4 than did mice vaccinated with an H7N2 reassortant virus (2). The relative immunogenic inferiority of N2 antigen when administered in vaccine virus containing H3, the HA previously encountered by animals sequentially infected with H3N2 heterovariants, cannot be explained by differential primary immunogenicity of the H3N2 or H7N2 vaccines. Studies in unprimed mice showed that, when given at equal dosage, the two vaccines induced comparable levels of NA inhibiting (anti-N2) antibody and homologous HA inhibiting antibody (2), and the present studies have shown equivalent N2-specific response of splenocytes *in vitro*. A likely explanation for this phenomenon posits a different immunologic response at the level of recognition and presentation of NA

antigen in association with a novel HA antigen. The studies presented here provide evidence for a difference in the recognition and processing of vaccine viral particles in mice primed to H3 antigen. H3N2 viral particles apparently are captured preferentially by H3 memory B cells, which action reduces the opportunity for N2 to unite with B- or T-cell receptors that would result in N2 antibody production, whereas recognition of H7N2 viral particles by N2 memory B cells is unimpaired by concomitant H3 recognition. In either case, processed viral antigens can then be presented to immune T cells, resulting in the activation of both T and B cells.

In the present study we also have shown that the proliferative response of T cells from H7N2-injected animals mixed with naive B cells stimulated by H6N2 virus was superior to that of any other T-cell group (Table 5). However, when H3N1 virus was used to stimulate the same T cells mixed with naive B cells, the resulting proliferative response was significantly inferior to the proliferation of T cells from H3N2-vaccinated mice mixed with naive B cells. These results are consistent with our previous finding of reduced generation of helper T cells specific for N2 antigen in animals sequentially infected by H3N2 heterovariant viruses and then injected with H3N2 vaccine. But now we have demonstrated both an expansion of N2-specific B cells in mice administered a booster of H7N2 vaccine and an expanded H3-specific B-cell population in H3N2-vaccinated mice. Furthermore, the present *in vitro* system has enabled us to segregate and assess independently the role of B and T cells in immunization by H3N2 influenza viruses and has resulted in a clear definition of the contribution both of primed B cells and of helper T memory cells in the mediation of intravirionic antigenic competition.

Differences in proliferative responses among B/T cell mixtures cannot be explained as the effect of unique interfacing among cells removed from the same animal (Table 4) or among naive cells (Tables 5 and 6). When B cells from H3N2-boosted mice were mixed with T cells from the other groups and stimulated with H3N1, it is clear that, regardless of the T-cell group, they always yielded a better response than was observed with any other B-cell group. Similarly, the proliferative response induced by H6N2 virus with B cells from H7N2-vaccinated mice was always superior to the proliferation of any other group, regardless of the T-cell group used. While this effect was seen with both H3N1-stimulated B cells as well as H6N2-stimulated B cells of H7N2-vaccinated mice, the effect was more pronounced with H6N2. This probably resulted from the fact that all B-cell groups with the exception of those from naive animals were primed to H3 antigen, while B cells from H7N2-vaccinated mice were better primed to N2 antigen. This point is supported by the observation that B cells from animals that were not injected with influenza A virus vaccine antigens after sequential infection induced significantly greater T-cell proliferation in response to H3N1 virus than occurred with H6N2 virus. Also, T cells from H3N2-vaccinated mice always responded better to H3N1 than did other T cells, regardless of the B cells used in antigen presentation, and T cells from H7N2-vaccinated animals were always better responders to H6N2 virus, regardless of the B cells used. However, an interfacing effect was observed in homologous cell mixtures; the greatest proliferative response to H6N2 virus was in homologous B/T-cell mixtures from H7N2-vaccinated mice, and the greatest response to H3N1 virus was in B/T-cell mixtures from mice given H3N2 vaccine. Taken together, all of these data provide evidence that both B cells and T cells contribute to intravirionic antigenic competition between influenza virus surface glycoproteins.

The model we propose for intravirionic antigenic competition assumes that antigen presentation by macrophages probably precedes or coincides with antigen presentation by B cells. This assumption is based on recent work indicating that B cells present antigen efficiently only in secondary immune responses or late in the primary response (11–14). We postulate that, in the first recognition of influenza virus antigens by the immune system, they are probably engulfed by macrophages or dendritic cells, processed, and presented to T cells. Because HA is found in greater molar amounts on the virion surface than NA (15) and because the degree and direction of antigenic competition are dependent on the relative amounts of competing immunogens, the resulting immune response is relatively HA-skewed. If, however, infection (or vaccination) occurs with a virus containing a previously encountered NA and an HA to which the host is immunologically naive, NA-specific memory B cells, expanded by previous exposure, more efficiently capture viral particles and present antigen to memory T cells, resulting in B- and T-cell activation. Resting B cells recognizing the novel HA are probably competitively blocked by the more efficient, activated NA-specific B cells.

Our studies have direct application in defining the complex immunologic response in influenza and reinforce earlier proposals (16) for an unconventional approach to immunization that utilizes viral NA as a primary immunogen (16, 17). To the degree that antibody to the viral NA is important in immunity to influenza (18), the repeated damping of response to this antigen in favor of HA with each reinfection intrinsically and inevitably prohibits the attainment of balanced immunity to both HA and NA antigens. The situation is analogous to the well-documented phenomenon of "original antigenic sin" (19)—a perversion of the anamnestic response in which primed response to conserved HA epitopes outstrips and may interfere with primary response to newly mutated antigenic sites on the HA of the notoriously changeable influenza A virus. Thus, both original antigenic sin and intravirionic HA–NA antigenic competition may operate to compromise the attainment of immunity to influenza by fostering inappropriate immunologic response.

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